

=> index bioscience medicine

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 17:26:00 ON 06 AUG 2006

71 FILES IN THE FILE LIST IN STNINDEX

=> s ((beta-carotene adj ketolase) or (carotene adj ketolase)or ketolase)

9 FILE AGRICOLA  
6 FILE AQUASCI  
12 FILE BIOENG  
185 FILE BIOSIS  
41 FILE BIOTECHABS  
41 FILE BIOTECHDS  
11 FILE BIOTECHNO  
6 FILE CABA  
94 FILE CAPLUS  
2 FILE CEABA-VTB  
2 FILE DDFB  
1077 FILE DGENE  
2 FILE DISSABS  
2 FILE DRUGB  
1 FILE DRUGU  
2 FILE EMBAL  
22 FILE EMBASE  
28 FILE ESBIODASE  
15 FILE FROSTI  
33 FILES SEARCHED...  
13 FILE FSTA  
115 FILE GENBANK  
26 FILE IFIPAT  
2 FILE JCST-EPLUS  
18 FILE LIFESCI  
29 FILE MEDLINE  
8 FILE PASCAL  
20 FILE PCTGEN  
1 FILE PROMT  
36 FILE SCISEARCH  
28 FILE TOXCENTER  
82 FILE USPATFULL  
17 FILE USPAT2  
1 FILE WATER  
33 FILE WPIDS  
1 FILE WPIFV  
33 FILE WPINDEX  
1 FILE NAPRALERT  
1 FILE NLDB

38 FILES HAVE ONE OR MORE ANSWERS, 71 FILES SEARCHED IN STNINDEX

L1 QUE ((BETA-CAROTENE ADJ KETOLASE) OR (CAROTENE ADJ KETOLASE) OR KETOLASE)

=> d rank

F1 1077 DGENE  
F2 185 BIOSIS  
F3 115 GENBANK  
F4 94 CAPLUS  
F5 82 USPATFULL  
F6 41 BIOTECHABS  
F7 41 BIOTECHDS  
F8 36 SCISEARCH  
F9 33 WPIDS  
F10 33 WPINDEX  
F11 29 MEDLINE  
F12 28 ESBIODASE

F13 28 TOXCENTER  
F14 26 IFIPAT  
F15 22 EMBASE  
F16 20 PCTGEN  
F17 18 LIFESCI  
F18 17 USPAT2  
F19 15 FROSTI  
F20 13 FSTA  
F21 12 BIOENG  
F22 11 BIOTECHNO  
F23 9 AGRICOLA  
F24 8 PASCAL  
F25 6 AQUASCI  
F26 6 CABA  
F27 2 CEABA-VTB  
F28 2 DDFB  
F29 2 DISSABS  
F30 2 DRUGB  
F31 2 EMBAL  
F32 2 JICST-EPLUS  
F33 1 DRUGU  
F34 1 PROMT  
F35 1 WATER  
F36 1 WPIFV  
F37 1 NAPRALERT  
F38 1 NLDB

=> file f2, f4-f15

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COPYRIGHT (C) 2006 IFI CLAIMS(R) Patent Services (IFI)

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=> s L1

L2 604 L1

=> s (microorganism or organism or phaffia) (s) L2  
L3 77 (MICROORGANISM OR ORGANISM OR PHAFFIA) (S) L2

=> s canthaxanthin(s)L3  
L4 23 CANTHAXANTHIN(S) L3

=> dup rem L4  
PROCESSING COMPLETED FOR L4  
L5 17 DUP REM L4 (6 DUPLICATES REMOVED)

=> d ibib abs L5 1-17

L5 ANSWER 1 OF 17 USPATFULL on STN DUPLICATE 1  
ACCESSION NUMBER: 2006:167050 USPATFULL <<LOGINID::20060806>>  
TITLE: Production of canthaxanthin by phaffia  
INVENTOR(S): Hoshino, Tatsuo, Kamakura-shi, JAPAN  
Ojima, Kazuyuki, Fujisawa-shi, JAPAN  
Setoguchi, Yutaka, Fujisawa-shi, JAPAN

NUMBER KIND DATE

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PATENT INFORMATION: US 2006141557 A1 20060629  
APPLICATION INFO.: US 2003-528846 A1 20030916 (10)  
WO 2003-EP10294 20030916  
20060209 PCT 371 date

NUMBER DATE

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PRIORITY INFORMATION: EP 2002-21600 20020927  
DOCUMENT TYPE: Utility  
FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: Stephen M Haracz, Bryan Cave, 1290 Avenue of the  
Americas, New York, NY, 10104, US  
NUMBER OF CLAIMS: 7  
EXEMPLARY CLAIM: 1  
LINE COUNT: 449  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB Disclosed is a process for producing \*\*\*canthaxanthin\*\*\* and  
echinenone which comprises cultivating a recombinant  
\*\*\*microorganism\*\*\* which is expressing a .beta.-casotene  
\*\*\*ketolase\*\*\* gene and belonging to the genus Xanthophyllomyces (  
\*\*\*Phaffia\*\*\* ) in an aqueous nutrient medium under aerobic conditions,  
and isolating the resulted carotenoids from the cells of said  
recombinant \*\*\*microorganism\*\*\* or from the cultured broth.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 2 OF 17 USPATFULL on STN  
ACCESSION NUMBER: 2006:132801 USPATFULL <<LOGINID::20060806>>  
TITLE: Process for preparing ketocarotenoids in genetically  
modified organisms  
INVENTOR(S): Sauer, Matt, Quedlinburg, GERMANY, FEDERAL REPUBLIC OF  
Flachmann, Ralf, Quedlingburg, GERMANY, FEDERAL  
REPUBLIC OF  
Klebsattel, Martin, Quedlingburg, GERMANY, FEDERAL  
REPUBLIC OF  
Schopfer, Christel Renate, Quedlingburg, GERMANY,  
FEDERAL REPUBLIC OF  
PATENT ASSIGNEE(S): SunGene GmbH & Co.KGaA, Gatersleben, GERMANY, FEDERAL  
REPUBLIC OF, 06466 (non-U.S. corporation)

NUMBER KIND DATE

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PATENT INFORMATION: US 2006112451 A1 20060525  
APPLICATION INFO.: US 2003-524827 A1 20030818 (10)  
WO 2003-EP9106 20030818  
20050218 PCT 371 date

NUMBER DATE

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PRIORITY INFORMATION: DE 2002-10238980 20020820

DE 2002-10238978 20020820

DE 2002-10238979 20020820

DE 2002-10253112 20021113

DE 2002-10258971 20021216

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: CONNOLLY BOVE LODGE & HUTZ, LLP, P O BOX 2207,  
WILMINGTON, DE, 19899, US

NUMBER OF CLAIMS: 46

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 18 Drawing Page(s)

LINE COUNT: 5572

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a process for preparing ketocarotenoids  
by cultivation of genetically modified organisms which, compared with  
the wild type, have a modified ketolase activity, to the genetically  
modified organisms, and to the use thereof as human and animal foods and  
for producing ketocarotenoid extracts.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 3 OF 17 USPATFULL on STN

ACCESSION NUMBER: 2006:62341 USPATFULL <<LOGINID::20060806>>

TITLE: Method for producing ketocarotenoids by cultivating  
genetically modified organisms

INVENTOR(S): Steiger, Sabine, Darmstadt, GERMANY, FEDERAL REPUBLIC  
OF  
Sandmann, Gerhard, Oberusel, GERMANY, FEDERAL REPUBLIC  
OF

NUMBER KIND DATE

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PRIORITY INFORMATION: US 2006053513 A1 20060309

APPLICATION INFO.: US 2003-541513 A1 20031224 (10)

WO 2003-EP14876 20031224

20050708 PCT 371 date

NUMBER DATE

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PRIORITY INFORMATION: DE 2003-103 20030109

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: CONNOLLY BOVE LODGE & HUTZ, LLP, P O BOX 2207,  
WILMINGTON, DE, 19899, US

NUMBER OF CLAIMS: 43

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 4 Drawing Page(s)

LINE COUNT: 2421

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a process for preparing ketocarotenoids  
by cultivation of genetically modified organisms which, compared with  
the wild type, have a modified ketolase activity, to the genetically  
modified organisms, and to the use thereof as human and animal foods and  
for producing ketocarotenoid extracts.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 4 OF 17 USPATFULL on STN

ACCESSION NUMBER: 2006:41436 USPATFULL <<LOGINID::20060806>>

TITLE: Natural promoters for gene expression in C1  
metabolizing bacteria

INVENTOR(S): Dicosimo, Deana J., Rockland, DE, UNITED STATES  
Ni, Hao, Newark, DE, UNITED STATES  
Picataggio, Stephen K., Landenberg, PA, UNITED STATES  
Seip, John E., Alloway, NJ, UNITED STATES  
Ye, Rick W., Hockessin, DE, UNITED STATES  
Wang, Tao, Hockessin, DE, UNITED STATES

NUMBER KIND DATE

PATENT INFORMATION: US 2006036088 A1 20060216  
APPLICATION INFO.: US 2005-251304 A1 20051014 (11)  
RELATED APPLN. INFO.: Division of Ser. No. US 2003-689200, filed on 20 Oct  
2003, PENDING

NUMBER DATE

PRIORITY INFORMATION: US 2002-419872P 20021021 (60)  
DOCUMENT TYPE: Utility  
FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: E I DU PONT DE NEMOURS AND COMPANY, LEGAL PATENT  
RECORDS CENTER, BARLEY MILL PLAZA 25/1128, 4417  
LANCASTER PIKE, WILMINGTON, DE, 19805, US  
NUMBER OF CLAIMS: 18  
EXEMPLARY CLAIM: 1-3  
NUMBER OF DRAWINGS: 3 Drawing Page(s)  
LINE COUNT: 2740  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB Genes have been identified in the Methylobacterium genome that are  
responsive to various metabolic and growth conditions. The identified  
responsiveness of these genes allows for the use of their promoters in  
regulated gene expression in C1 metabolizing bacteria. In particular,  
the hps promoter, which in its native state drives the expression of  
3-hexulose-6-phosphate synthase (HPS), was found to be useful for  
directing expression of heterologous coding regions (e.g., crtZ) in the  
obligate methanotroph Methylobacterium sp. 16a.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 17 USPATFULL on STN  
ACCESSION NUMBER: 2006:3946 USPATFULL <<LOGINID::20060806>>  
TITLE: Carotenoid ketolase genes with improved ketocarotenoid  
yield  
INVENTOR(S): Tang, Xiao-Song, Hockessin, DE, UNITED STATES  
Cheng, Qiong, Wilmington, DE, UNITED STATES  
Tao, Luan, Havertown, PA, UNITED STATES  
Shyr, Joanne Y., Newark, DE, UNITED STATES

NUMBER KIND DATE

PATENT INFORMATION: US 2006003403 A1 20060105  
APPLICATION INFO.: US 2005-147915 A1 20050608 (11)

NUMBER DATE

PRIORITY INFORMATION: US 2004-577970P 20040608 (60)  
DOCUMENT TYPE: Utility  
FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: E I DU PONT DE NEMOURS AND COMPANY, LEGAL PATENT  
RECORDS CENTER, BARLEY MILL PLAZA 25/1128, 4417  
LANCASTER PIKE, WILMINGTON, DE, 19805, US  
NUMBER OF CLAIMS: 29  
EXEMPLARY CLAIM: 1  
NUMBER OF DRAWINGS: 2 Drawing Page(s)  
LINE COUNT: 2138  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB Protein engineered CrtO ketolases are provided having increased  
carotenoid ketolase activity. Methods using the present CrtO ketolases  
are also provided for increasing ketocarotenoid production in suitable  
production hosts.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
DUPLICATE 2  
ACCESSION NUMBER: 2005-27515 BIOTECHDS <<LOGINID::20060806>>  
TITLE: Stably expressing a nucleic acid molecule in one carbon  
metabolizing microorganism comprises integrating nucleic acid

molecule in tig region of genome of microorganism;  
vector-mediated gene transfer and expression in host cell  
for strain improvement and carotenoid compound production

AUTHOR: MILLER E S; YE R W

PATENT ASSIGNEE: DU PONT DE NEMOURS and CO E I

PATENT INFO: WO 2005087942 22 Sep 2005

APPLICATION INFO: WO 2005-US7120 4 Mar 2005

PRIORITY INFO: US 2004-550385 5 Mar 2004; US 2004-550385 5 Mar 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-658903 [67]

AN 2005-27515 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid molecule is stably expressed in a one carbon metabolizing \*\*\*microorganism\*\*\* by providing a one carbon metabolizing \*\*\*microorganism\*\*\* having a tig region in the genome; integrating nucleic acid molecule(s) into the tig region of the genome of the metabolizing \*\*\*microorganism\*\*\*; and growing the metabolizing \*\*\*microorganism\*\*\* under conditions where the nucleic acid molecule is stably-expressed, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (A) production of a carotenoid compound comprising providing one carbon (1C) metabolizing \*\*\*microorganism\*\*\* comprising a gene cluster comprising genes encoding the carotenoid biosynthetic pathway inserted into the tig region of the genome; contacting the metabolizing \*\*\*microorganism\*\*\* with 1C carbon substrate from methane and/or methanol under conditions where the gene cluster is expressed and a carotenoid compound is produced; and optionally recovering the carotenoid compound; (B) a 1C metabolizing \*\*\*microorganism\*\*\* comprising a nucleic acid molecule integrated in the tig region of the genome; and (C) identifying an integration site in a genome for high level expression of a nucleic acid molecule \*\*\*microorganism\*\*\* comprising: (a) providing an integration vector comprising a gene cluster encoding the following enzymes: geranylgeranyl pyrophosphate synthase, zeaxanthin glucosyl transferase; lycopene cyclase, phytoene desaturase, phytoene synthase, 13-carotene hydroxylase, 13-carotene \*\*\*ketolase\*\*\*, and isopentenyl diphosphate isomerase, to facilitate the integration of the gene cluster in to the genome of a \*\*\*microorganism\*\*\*; (b) contacting the integration vector with a \*\*\*microorganism\*\*\* under conditions that allow for random integration of the gene cluster into the \*\*\*microorganism\*\*\* genome to create random transformants; (c) screening the random transformants for expression of the gene cluster on the basis of the production of a 40C carotenoid; and (d) identifying sites of integration of the gene cluster into the genome of the random transformants.

BIOTECHNOLOGY - Preferred Method: The nucleic acid molecule is transcribed using the tig promoter. It is operably integrated. Multiple unlinked genes are integrated at different positions within the tig region. The nucleic acid molecule is integrated into the tig region downstream of the tig promoter or of any gene of the tig region. It can be integrated downstream of the tig open or ion open reading frame, or downstream of the clpP open reading frame. Preferred Nucleic Acid: The nucleic acid molecule lacks an antibiotic selection marker. The nucleic acid molecule comprises multiple tandem genes in a single fragment. It is a gene. The tig region is defined according to a fully defined 9010 base pairs sequence given in the specification. The nucleic acid molecule is genes encoding transaldolase, fructose biphosphate aldolase, keto deoxy phosphogluconate aldolase, phosphoglucomutase, glucose-6-phosphate isomerase, phosphofructokinase, 6-phosphogluconate dehydratase, 6-phosphogluconate-6-phosphate-1 dehydrogenase, dxs, dxr, ispA, ispD, ispE, ispF, crtE, crtX, crtY, crtI, crtB, crtZ, crtD, crtO, crtW, crtidi, genes encoding limonene synthase, ugp, gumD, wza, espB, espM, waaE, espV, gumH, genes encoding glycosyltransferase genes, aroG, aroB, aroQ, aroE, aroK, 5-enolpyruvylshikimate-3phosphate synthase, aroC, trpE, trpD, trpC, trpB, pheA, tyrAc, pds, phaC, phaE, efe, pdc, adh, pinene synthase, bornyl synthase, phellandrene synthase, cineole synthase, sabinene synthase, or taxadiene synthase. It encodes at least one enzyme in the carotenoid biosynthetic pathway. The enzyme in the carotenoid biosynthetic pathway is geranylgeranyl pyrophosphate synthase, zeaxanthin glucosyl transferase, lycopene cyclase, phytoene desaturase, phytoene synthase, beta-carotene hydroxylase, beta-carotene \*\*\*ketolase\*\*\*, or

isopentenyl diphosphate isomerase. Preferred \*\*\*Microorganism\*\*\* : The metabolizing \*\*\*microorganism\*\*\* is methanotrophs or methylotrophs. It can be Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocystis, Methylobacterium, Methanomonas, Methylophilus, Methylobacillus, Methylobacterium, Hyphomicrobium, Xanthobacter, Bacillus, Paracoccus, Nocardia, Arthrobacter, Rhodospseudomonas, or Pseudomonas. It is Methylomonas 16a. The metabolizing \*\*\*microorganism\*\*\* has the ATCC designation ATCC PTA 2402. Preferred Carotenoid: The carotenoid compound is antheraxanthin, adonixanthin, astaxanthin, canthaxanthin, aanthaxanthin, capsorubrin, alpha-cryptoxanthin alpha-carotene, beta-carotene, epsilon-carotene, echinenone, gamma-carotene, zeta-carotene, alpha-cryptoxanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene, lactucaxanthin, lutein, lycopene, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, uriolide, uriolide acetate, violaxanthin, zeaxanthin-beta-diglucoside, zeaxanthin, or \*\*\*canthaxanthin\*\*\* .

USE - For stably expressing a nucleic acid molecule in 1C metabolizing \*\*\*microorganism\*\*\* , useful for production of a carotenoid compound (claimed).

ADVANTAGE - The invention results in high level and stable production of 40C carotenoids.

EXAMPLE - The growth of Methylomonas sp. 16a for tri-parental mating initiated with the inoculation of -80degreesC frozen stock culture into 20 ml ammonium liquid medium containing 25% methane. The culture was grown at 30degreesC with aeration. This saturated culture was used to inoculate 100 ml of fresh ammonium liquid medium containing 25% methane. The 100 ml culture was grown at 30degreesC with aeration until the culture reached an OD600 between 0.7-0.8. The Methylomonas cell pellets were re-suspended. The re-suspended Methylomonas cells were used to re-suspend the combined Escherichia coli and helper cell pellets. The cell suspension was spotted on agar plates containing 0.05% yeast extract. The plates were incubated at 30degreesC for 3 days in a jar containing 25% methane. The cells from the cultures were inoculated in ammonium liquid medium and 25% methane and grown overnight at 30degreesC with aeration. The cultures were monitored for Escherichia coli growth by plating on agar plates to verify the elimination of the Escherichia coli. (136 pages)

L5 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:1314197 CAPLUS <<LOGINID::20060806>>

DOCUMENT NUMBER: 144:47255

TITLE: Method of producing astaxanthin or its metabolic intermediates in recombinant microorganism or transgenic plants introduced with the Brevundimonas genes for carotenoid ketolase and carotenoid hydroxylase

INVENTOR(S): Choi, Seon-Kang, Misawa, Norihiko

PATENT ASSIGNEE(S): Marine Biotechnology Institute Co., Ltd., Japan

SOURCE: PCT Int. Appl., 45 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005118812	A1	20051215	WO 2005-JP9609	20050526
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT,				

RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,  
MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: JP 2004-166625 A 20040604

AB The genes (crtW, crtY, crtI, crtB, crtE, crtZ, idi and five other ORFs) involved in carotenoid metab. have been cloned from the genome of *Brevundimonas* sp strain SD-212. The crtW gene (for .beta.-ionone ring-4-ketolase) among them has been inserted into a vector and introduced into host microbial or plant organisms for producing astaxanthin or related pigment metabolites. The recombinant *E. coli* JM109 strains bearing *Brevundimonas* crtW were demonstrated that they can produce significant amt. of astaxanthin together with the other metabolic intermediates including zeaxanthin, 3'-hydroxyechinenone, 3-hydroxyechinenone, lycopene, and adonixanthin. The crtZ gene (for .beta.-ionone ring-3-hydroxylase) has been inserted into a vector and introduced into host microbial or plant organisms for producing astaxanthin or related pigment metabolites. The recombinant *E. coli* JM109 strains bearing *Brevundimonas* crtZ were demonstrated that they can produce significant amt. of astaxanthin together with the other metabolic intermediates including canthaxanthin and adonirubin and adonixanthin. The establishment of the astaxanthin prodn. method using recombinant organisms (microbial or plant) enables the large scale provision of the biol. active carotenoids in the health food industries.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2006-03315 BIOTECHDS <<LOGINID::20060806>>

TITLE: Novel isolated nucleic acid molecule encoding mutant carotenoid ketolase, useful for producing recombinant microorganisms producing ketocarotenoid such as canthaxanthin, astaxanthin, adonixanthin, adonirubin, echinenone, and myxobactone; involving vector-mediated gene transfer and expression in *Escherichia coli*, *Methylobacter* sp., yeast, fungus, alga or green plant for use as a food-additive, in fish and fowl pigmentation, feedstuff, pharmaceutical and cosmetic industries and transgenic animal and transgenic plant construction

AUTHOR: TANG X; CHENG Q; TAO L; SHYR J Y

PATENT ASSIGNEE: DU PONT DE NEMOURS and CO E I

PATENT INFO: WO 2005121352 22 Dec 2005

APPLICATION INFO: WO 2005-US20411 8 Jun 2005

PRIORITY INFO: US 2004-577970 8 Jun 2004; US 2004-577970 8 Jun 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2006-048178 [05]

AN 2006-03315 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid (N1) molecule encoding a mutant carotenoid \*\*\*ketolase\*\*\* having a fully defined 532 amino acid (SEQ ID No. 2) sequence given in the specification, and comprising an amino acid replacement at one or more of 8 specified positions, is new.

DETAILED DESCRIPTION - An isolated nucleic acid (N1) molecule encodes a mutant carotenoid \*\*\*ketolase\*\*\* having a fully defined 532 amino acid (SEQ ID No. 2) sequence given in the specification, and comprising one or more of: (a) replacement of threonine 121 with alanine; (b) replacement of methionine 142 with leucine; (c) replacement of alanine 164 with valine; (d) replacement of isoleucine 183 with valine; (e) replacement of threonine 304 with lysine; (f) replacement of arginine 339 with glutamine; (g) replacement of arginine 519 with tryptophan; and (h) replacement of glutamine 524 with leucine or arginine. (N1) may also encode an amino acid sequence selected from SEQ ID NOs 14-52 (even SEQ ID NOs), where SEQ ID NO:16 comprises 535 amino acids, and the other SEQ ID NOs comprise 532 amino acids. INDEPENDENT CLAIMS are also included for: (1) a polypeptide (P1) encoded by (N1), having an amino acid sequence chosen from SEQ ID No. 14-52 (even SEQ ID numbers); (2) a chimeric gene comprising (N1) operably linked to suitable regulatory sequences; and (3) a transformed host cell (I) comprising (N1); (4) producing cyclic ketocarotenoid compounds, comprising: (a) providing a host producing monocyclic or bicyclic caretenoids; (b) transforming the host cell with



the (N1); (c) growing the transformed host cell under conditions where a cyclic ketocarotenoid is produced; and (d) optionally isolating the ketocarotenoid; and (4) altering cyclic ketocarotenoid biosynthesis in an \*\*\*organism\*\*\* by introducing (N1) into the host cell.

**BIOTECHNOLOGY - Preferred Nucleic Acid: (N1)** is chosen from one of 19 fully defined 1599 base pair sequences (SEQ ID No. 13, 17-51 (odd SEQ ID numbers)) given in the specification, and having a fully defined 1608 base pair (SEQ ID No. 16) sequence given in the specification. Preferred Host: (I) is chosen from bacteria, yeast, filamentous fungi, algae and green plants, preferably from *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, \*\*\**Phaffia*\*\*\*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Erythrobacter*, *Chloroborium*, *Chromatium*, *Flavobacterium*, *Cytophaga*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Deinococcus*, *Escherichia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Methanomonas*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella* and *Myxococcus*. (I) is preferably chosen from *Escherichia* and *Methylomonas*, most preferably *Escherichia coli* and *Methylomonas* sp. 16a (ATCC PTA-2402). (I) is chosen from soybean, rapeseed, pepper, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees and forage grasses. Preferred Method: In producing cyclic ketocarotenoid compounds the compounds are selected from \*\*\*canthaxanthin\*\*\*, astaxanthin, adonixanthin, adonirubin, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, 4-keto-gamma-carotene, 4-keto-rubixanthin, 4-keto-torulene, 3-hydroxy-4-keto-torulene, deoxyflexixanthin, and myxobactone. The monocyclic or bicyclic carotenoids are selected from beta-carotene, gamma-carotene, zeaxanthin, rubixanthin, echinenone, and torulene. The host cell is selected from bacteria, yeast, filamentous fungi, algae, and green plants. In altering cyclic ketocarotenoid biosynthesis in an \*\*\*organism\*\*\*, (N1) is upregulated, over-expressed on a multicopy plasmid and/or operably linked to an inducible or regulated promoter. (N1) may be down-regulated by expression in an antisense orientation or by insertion of foreign DNA into the coding region.

**USE - (N1)** is useful for transforming a host cell, where the transformed cell (I) is useful for producing cyclic ketocarotenoid compounds (claimed). The ketocarotenoids produced are useful in pharmaceuticals, food supplements, fish and poultry pigmentation, electro-optic applications, animal feed additives, and as colorants in cosmetics. (N1) is useful for producing recombinant organisms having the ability to produce more amounts of ketocarotenoid compounds, and for producing transgenic plants having the ability to express the microbial protein.

**ADVANTAGE - (N1)** enables production of recombinant microorganisms capable of producing ketocarotenoid, and encodes carotenoid ketolases having improved activity for ketocarotenoid production.

**EXAMPLE -** Plasmids containing the mutant genes in WS210 host *Escherichia coli* cells were incorporated into *Methylomonas* 16a through conjugation. *Methylomonas* sp. 16a (ATCC PTA-2402) cells expressing the mutant crtO genes were then grown in nitrate liquid medium having nitroacetic acid, copper chloride, ferric chloride, manganese chloride, copper chloride, zinc chloride, borate, sodium molybdate and nickel chloride. The nitrate was replaced with 15 mM ammonium chloride. The amounts of \*\*\*canthaxanthin\*\*\* produced in the medium were analyzed by HPLC. The standard gas phase for cultivation contains 25% methane in air. *Methylomonas* 16a strains expressing various crtO mutants were grown in serum stoppered Wheaton bottles using a gas/liquid ratio of at least 8:1 at 30degreesC with constant shaking. Percentage yield of \*\*\*canthaxanthin\*\*\* in *Methylomonas* strains containing CrtO319 (starting genes), CrtO303 (starting genes), CrtO320 (starting genes), 320M4019, 320SHU001 or 320SHU019 was found to be 3-9%, 7-15%, 15-21%, 38-40%, 45-53% and 44-51%, respectively. Results indicated that the mutant crtO genes encode carotenoid ketolases that exhibits improved activity for \*\*\*canthaxanthin\*\*\* production.(163 pages)

ACCESSION NUMBER: 2005-22605 BIOTECHDS <<LOGINID::20060806>>

TITLE: New genetically engineered bacterium having a disruption in one or more *gdhA*, *gpmB* *aceE*, *ppc*, *talB*, *fdhF*, *yjiD*, *hnr* or *yjfP* genes, useful for overexpressing genes impacting carotene biosynthesis and enhancing carotenoid production; involving vector-mediated gene transfer and expression in host cell

AUTHOR: STEPHANOPOULOS G; ALPER H S; JIN Y

PATENT ASSIGNEE: MASSACHUSETTS INST TECHNOLOGY

PATENT INFO: WO 2005062923 14 Jul 2005

APPLICATION INFO: WO 2004-US43295 23 Dec 2004

PRIORITY INFO: US 2004-609246 14 Sep 2004; US 2003-531996 24 Dec 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-522448 [53]

AN 2005-22605 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - A genetically engineered bacterium comprising a disruption in one or more *gdhA*, *gpmB* *aceE*, *ppc*, *talB*, *fdhF*, *yjiD*, *hnr* or *yjfP* genes, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a carotenoid- producing bacterium, comprising one or more inhibitors of *gdhA*, *gpmB* *aceE*, *ppc*, *talB*, *fdhF*, *yjiD*, *hnr* or *yjfP* gene activity, or their combination, where use of the one or more inhibitors results in enhanced carotenoid production in the bacterium, as compared to wildtype; (2) a method for production of carotenoids, comprising genetically disrupting a *gdhA*, *gpmB* *aceE*, *ppc*, *talB*, *fdhF*, *yjiD*, *hnr* or *yjfP* gene, or their combination in a cell comprising genes involved in the carotenoid biosynthetic pathway, and isolating carotenoids from the cell, and producing carotenoids; (3) a method for enhanced production of carotenoids, comprising contacting a cell comprising genes involved in the carotenoid biosynthetic pathway with one or more inhibitors of *gdhA*, *gpmB*, *aceE*, *ppc*, *talB*, *fdhF*, *yjiD*, *hnr* or *yjfP* gene expression or function, or their combination, and isolating carotenoids from the cell, and enhancing production of carotenoids; (4) a cell genetically engineered to over-expresses one or more *dxs*, *idi*, *yjiD*, *rpoS*, *torC*, *appY*, *ydgK*, *yeiA*, *yedR*, *tort*, *arcB*, *yggT*, *purDH*, or *yfjN* genes; (5) a method of determining optimized production of a metabolite, comprising constructing a flux balance analysis model, applying constraints to the flux balance analysis model comprising maximizing cell growth yield subject to a minimization of metabolic adjustment alteration, conducting in silico gene knockout simulations for all genes in the \*\*\*organism\*\*\*'s genome, where a flux profile comprising a deletion of a knocked out gene from the stoichiometry matrix is calculated for the gene knockout simulations, and conducting in silico gene knockout simulations of all possible pairs or triplets of genes in the \*\*\*organism\*\*\*'s genome, where a flux profile comprising the deletions of genes from the stoichiometric matrix is calculated for the gene knockout simulations, and selecting gene targets on the basis of enhanced flux profiles as a measure of optimal production of the metabolite, and determining optimized production of a metabolite; (6) a method of identifying genes involved in optimized production of a carotenoid, comprising constructing a flux balance analysis model, applying constraints to the flux balance analysis model comprising maximizing cell growth yield subject to a minimization of metabolic adjustment alteration, conducting in silico gene knockout simulations for all genes in the \*\*\*organism\*\*\*'s genome, where a flux profile comprising a deletion of a knocked out gene from the stoichiometry matrix is calculated for the gene knockout simulations, conducting in silico gene knockout simulations of all possible pairs or triplets of genes in the \*\*\*organism\*\*\*'s genome, where a flux profile comprising the deletions of genes from the stoichiometric matrix is calculated for the gene knockout simulations, selecting gene targets on the basis of enhanced flux profiles as a measure of optimal production of the metabolite, contacting cells comprising genes involved in the carotenoid biosynthetic pathway with a library of transposon mutagenized genes, selecting cells with enhanced carotenoid synthesis or production, and identifying mutagenized sequences, and inhibiting or abrogating gene expression of the gene targets identified, and their combinations, in a cell comprising genes involved in the carotenoid biosynthetic pathway, and determining

carotenoid production in the cell, and identifying genes involved in optimized production of a carotenoid; and (7) a genetically engineered cell with enhanced lycopene production, where the cell is genetically disrupted for a gene or genes identified in the method of (6), and where the cell is suppressed or inhibited for expression of a gene or genes identified in the method of (6).

**BIOTECHNOLOGY - Preferred Bacterium:** The genetically engineered bacterium comprises a disruption in the genes *gdhA* and *gpmB*, genes *gdhA* and *aceE*, genes *gdhA* and *ppc*, genes *gdhA* and *fdhF*, or genes *gdhA* and *talB*, and further comprises a disruption in the *fdhF* or *talB* gene, and additionally comprises a disruption in the genes *gdhA*, *aceE* and *fdhF*, genes *gdhA* and *yjfP*, genes *gdhA*, *aceE* and *yjfP*, genes *gdhA*, *aceE*, *fdhF* and *yjfP*, genes *gpmB* and *yjiD*, genes *gdhA*, *aceE*, and *yjiD*, genes *gdhA*, *gpmB* and *yjiD*, genes *gdhA*, *aceE*, *hnr* and *yjfP*. The genetically engineered bacterium over-expresses *dxs*, *idi*, *yjiD*, *rpoS*, *torC*, *appY*, *ydgK*, *yeiA*, *yedR*, *tort*, *arcB*, *yggT*, *purDH*, *yfjN*, or their combination. The bacterium belongs to the *Escherichia*, *Methylobacter*, *Methylobacter*, *Methylobacter*, and *Methylobacter*, *Erwinia*, *Haematococcus*, *Rhodobacter*, *Myxococcus*, *Corynebacteria*, *Pseudomonas* or *Bacillus* genus. The bacterium further comprises an isopentenyl pyrophosphate isomerase (*Idi*), a farnesyl pyrophosphate synthetase (*IspA*), a geranyltransferase, an octoprenyl pyrophosphate synthase (*IspB*), a geranylgeranyl pyrophosphate (GGPP) synthase (*CrtE*), a phytoene synthase (*CrtB*), a phytoene desaturase (*CrtI*), a lycopene cyclase (*CrtY*), a beta-carotene hydroxylase (*CrtZ*), a zeaxanthin glucosyl transferase (*CrtX*), a beta-carotene \*\*\*ketolase\*\*\* (*CrtO*), or their combination. The one or more inhibitors in the carotenoid-producing bacterium comprise a nucleic acid. The presence of the one or more inhibitors in the bacterium results in enhanced carotenoid production of between 3 -50%, and suppresses or abrogates *gdhA* and *gpmB*, *gdhA* and *aceE*, *gdhA* and *ppc*, *gdhA* and *fdhF*, *gdhA* and *talB*, *fdhF*, *talB*, *gdhA*, *aceE* and *fdhF*, *gdhA* and *yjfP*, *gdhA*, *aceE* and *yjfP*, *gdhA*, *aceE*, *fdhF* and *yjfP*, *gpmB* and *yjiD*, *gdhA*, *aceE*, and *yjiD*, *gdhA*, *gpmB* and *yjiD*, *gdhA*, *aceE*, *hnr* and *yjfP*, *gdhA*, *aceE*, *hnr*, *yjfP* and *yjiD*, and/or *gdhA*, *gpmB* and *yjfP* gene expression or function. The bacterium also over-expresses *dxs*, *idi*, *yjiD*, *rpoS*, *torC*, *appY*, *ydgK*, *yeiA*, *yedR*, *tort*, *arcB*, *yggT*, *purDH*, *yfjN*, or their combination. The bacterium belongs to the *Escherichia*, *Methylobacter*, *Methylobacter*, *Methylobacter*, and *Methylobacter*, *Erwinia*, *Haematococcus*, *Rhodobacter*, *Myxococcus*, *Corynebacteria*, *Pseudomonas* or *Bacillus* genus. The bacterium further comprises an isopentenyl pyrophosphate isomerase (*Idi*), a farnesyl pyrophosphate synthetase (*IspA*), a geranyltransferase, an octoprenyl pyrophosphate synthase (*IspB*), a geranylgeranyl pyrophosphate (GGPP) synthase (*CrtE*), a phytoene synthase (*CrtB*), a phytoene desaturase (*CrtI*), a lycopene cyclase (*CrtY*), a beta-carotene hydroxylase (*CrtZ*), a zeaxanthin glucosyl transferase (*CrtX*), a beta-carotene \*\*\*ketolase\*\*\* (*CrtO*), or their combination.

**Preferred Method:** The genes in producing carotenoids are disrupted as mentioned in the bacterium cited above. The cell in enhanced production of carotenoids is contacted with one or more inhibitors of *gdhA* and *gpmB*, *gdhA* and *aceE*, *gdhA* and *ppc*, *gdhA* and *fdhF*, *gdhA* and *talB*, *fdhF*, *talB*, *gdhA*, *aceE* and *fdhF*, *gdhA* and *yjfP*, *gdhA*, *aceE* and *yjfP*, *gdhA*, *aceE*, *fdhF* and *yjfP*, *gpmB* and *yjiD*, *gdhA*, *aceE*, and *yjiD*, *gdhA*, *gpmB* and *yjiD*, *gdhA*, *aceE*, *hnr* and *yjfP*, *gdhA*, *aceE*, *hnr*, *yjfP* and *yjiD*, and/or *gdhA*, *gpmB* and *yjfP* expression or function. The one or more inhibitors comprise a nucleic acid. The cell is a bacterium. The method of enhancing production of carotenoids also comprises contacting a cell comprising genes involved in the carotenoid biosynthetic pathway with a plasmid comprising a *dxs*, *idi*, *yjiD*, *rpoS*, *torC*, *appY*, *ydgK*, *yeiA*, *yedR*, *tort*, *arcB*, *yggT*, *purDH*, *yfjN* gene, or their combination, culturing the cell where carotenoids are produced, and isolating carotenoids from the cell and enhancing production of carotenoids. The cell in any of the methods cited is a bacterium that belongs to the *Escherichia*, *Methylobacter*, *Methylobacter*, *Methylobacter*, and *Methylobacter*, *Erwinia*, *Haematococcus*, *Rhodobacter*, *Myxococcus*, *Corynebacteria*, *Pseudomonas* or *Bacillus* genus. The genes involved in the carotenoid biosynthetic pathway comprise an isopentenyl pyrophosphate isomerase (*Idi*), a farnesyl pyrophosphate synthetase (*IspA*), a geranyltransferase, an octoprenyl pyrophosphate synthase (*IspB*), a geranylgeranyl pyrophosphate (GGPP) synthase (*CrtE*), a phytoene synthase (*CrtB*), a phytoene desaturase (*CrtI*), a lycopene cyclase (*CrtY*), a beta-carotene hydroxylase (*CrtZ*), a zeaxanthin glucosyl

**EXAMPLE - Escherichia coli K12 PT5-dxs, PT5-idi, PT5-ispFD** was used as the lycopene expression strain when harboring the pAC-LYC plasmid containing the crtEB1 operon. Gene deletions were conducted using PCR product recombination using the pKD46 plasmid expressing the lambda red recombination system and pKD13 as the kan template for PCR.(111 pages)

AUTHOR: CHENG Q; TAO L; YAO H  
PATENT ASSIGNEE: DU PONT DE NEMOURS and CO E I

PATENT INFO: WO 2005062867 14 Jul 2005  
APPLICATION INFO: WO 2004-US43008 17 Dec 2004  
PRIORITY INFO: US 2003-531310 19 Dec 2003; US 2003-531310 19 Dec 2003  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2005-591251 [60]  
AN 2005-24440 BIOTECHDS <<LOGINID::20060806>>  
AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule encoding a carotenoid \*\*\*ketolase\*\*\* enzyme, is new. The nucleic acid molecule: (A) encodes any of the 3 sequences having 248, 259 or 256 amino acids fully defined in the specification (SEQ ID NOS: 2, 4 and 6, respectively); (B) hybridizes with (A) under the following wash conditions: 0.1XSSC, 0.1% SDS, 65degreesC, or (C) is complementary to (A) or (B).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a polypeptide encoded by the above nucleic acid molecule; (2) a chimeric gene comprising the isolated nucleic acid molecule operably linked to suitable regulatory sequences; (3) a transformed host cell comprising the chimeric gene; (4) obtaining a nucleic acid molecule encoding a carotenoid \*\*\*ketolase\*\*\* enzyme; (5) the product of (4); (6) production of cyclic ketocarotenoid compounds; (7) regulating cyclic ketocarotenoid biosynthesis in an \*\*\*organism\*\*\*; (8) a mutated gene encoding a carotenoid \*\*\*ketolase\*\*\* enzyme having an altered biological activity produced by a method comprising digesting a mixture of nucleotide sequences with restriction endonucleases, where the mixture comprises: a native carotenoid \*\*\*ketolase\*\*\* gene, a first population of nucleotide fragments which will hybridize to the native carotenoid \*\*\*ketolase\*\*\* gene, a second population of nucleotide fragments that will not hybridize to the native carotenoid \*\*\*ketolase\*\*\* gene, where a mixture of restriction fragments are produced; denaturing the mixture of restriction fragments; incubating the denatured mixture of restriction fragments with a polymerase; and repeating the denaturing and incubating steps, where a mutated carotenoid \*\*\*ketolase\*\*\* gene is produced encoding a protein having an altered biological activity; and (9) a method for increasing production of cyclic ketocarotenoid compounds.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid molecule comprises a sequence of 747, 780 or 771 bp fully defined in the specification (SEQ ID NOS: 1, 3 and 5, respectively). The nucleic acid molecule comprises a first nucleotide sequence encoding a carotenoid \*\*\*ketolase\*\*\* enzyme of at least 249 amino acids that has at least 75% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having SEQ ID NO: 2; or a first nucleotide sequence encoding a carotenoid \*\*\*ketolase\*\*\* enzyme of at least 260 amino acids that has at least 75% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having SEQ ID NO: 4 or 6; or a second nucleotide sequence comprising the complement of the first nucleotide sequence. Preferred Host Cell: The host cell is selected from bacteria, yeast, filamentous fungi, algae, and green plants. It is selected from *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, or *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Erythrobacter*, *Chlorobium*, *Chromatium*, *Flavobacterium*, *Cytophaga*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Deinococcus*, *Escherichia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, and *Myxococcus*. The host cell is a C1 metabolizing bacteria. In addition, the host cell is selected from soybean, rapeseed, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops, sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses. The carotenoid \*\*\*ketolase\*\*\* gene encodes a polypeptide having SEQ ID NO: 2, 4 or 6. Regulating cyclic ketocarotenoid biosynthesis in an \*\*\*organism\*\*\* comprises introducing into a host cell the above carotenoid \*\*\*ketolase\*\*\* gene under the control of suitable regulatory sequences and growing the host cell under conditions where the carotenoid \*\*\*ketolase\*\*\* gene is expressed and cyclic ketocarotenoid biosynthesis

is regulated. The carotenoid \*\*\*ketolase\*\*\* gene is upregulated. It is over-expressed on a multicopy plasmid and is operably linked to an inducible or regulated promoter. Alternatively, the carotenoid \*\*\*ketolase\*\*\* gene is down-regulated and is expressed in antisense orientation. The gene is disrupted by insertion of foreign DNA into the coding region. Increasing production of cyclic ketocarotenoid compounds comprises transforming the above host cell that produces cyclic carotenoids with a first gene selected from the genes cited above encoding a CrtW carotenoid \*\*\*ketolase\*\*\* enzyme, transforming the host cell with a second gene encoding a CrtW carotenoid \*\*\*ketolase\*\*\* enzyme, the second gene having less than 65% nucleic acid sequence identity when compared to the first gene; and growing the transformed host cell comprising the first gene and the second gene under conditions where the production of cyclic ketocarotenoid is increased relative to a transformed host cell only expressing either the first gene or the second gene. The transformed host cell is as mentioned above or is selected from *Spirulina*, *Haemotacoccus*, and *Dunalliella*. The nucleic acid molecule was prepared using standard isolation techniques. Preparation: Obtaining a nucleic acid molecule encoding a carotenoid \*\*\*ketolase\*\*\* enzyme comprises: (1) probing a genomic library with the nucleic acid molecule cited above, identifying a DNA clone that hybridizes with the above nucleic acid molecule under the following wash conditions: 0.1 X SSC, 0.1% SDS, 65degreesC and sequencing the genomic fragment that comprises the identified clone, where the sequenced genomic fragment encodes a carotenoid \*\*\*ketolase\*\*\* enzyme; (2) synthesizing at least one oligonucleotide primer corresponding to a portion of SEQ ID NO: 1, 3 or 5 and amplifying an insert present in a cloning vector using the above oligonucleotide primer, where the amplified insert encodes a carotenoid \*\*\*ketolase\*\*\* enzyme.

USE - Used for producing ketocarotenoids, such as \*\*\*canthaxanthin\*\*\* or astaxanthin, used as pharmaceuticals, food supplements, animal feed additives or colorants in cosmetics.

EXAMPLE - No relevant example given.(107 pages)

L5 ANSWER 11 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
 ACCESSION NUMBER: 2005-10841 BIOTECHDS <<LOGINID::20060806>>

TITLE: Preparation of ketocarotenoids, useful in foods and animal feeds, by growing genetically modified organism that has altered activity of ketolase and beta-cyclase; for food-additive and pigment preparation and as an antioxidant

AUTHOR: FLACHMANN R; SCHOPFER C R; HERBERS K; KUNZE I; SAUER M; KLEBSATTEL M; LUCK T; VOESTE D; PFEIFFER A

PATENT ASSIGNEE: SUNGENE GMBH and CO KGAA

PATENT INFO: WO 2005019467 3 Mar 2005

APPLICATION INFO: WO 2004-EP8623 31 Jul 2004

PRIORITY INFO: DE 2004-102004007622 17 Feb 2004; WO 2003-9101 18 Aug 2003

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 2005-202663 [21]

AN 2005-10841 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - Method for preparing ketocarotenoids (I) by growing a genetically modified \*\*\*organism\*\*\* (A) which, compared with the wild type, has altered activities of a \*\*\*ketolase\*\*\* (II) and a beta-cyclase (III), where the alteration in beta-cyclase activity is produced by an amino acid (aa) sequence (2; 498 aa), reproduced, or by its derivatives with at least 70% identity, formed by substitution, deletion or insertion.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for (A) in which \*\*\*ketolase\*\*\* and beta-cyclase activities are increased (or provided if not already present), where beta-cyclase activity is provided by expressing (2) or its derivatives as specified above.

BIOTECHNOLOGY - Preferred Organisms: These may already have some \*\*\*ketolase\*\*\* activity and this is increased by expression of a sequence (4; 329 aa) or its derivatives with at least 70% identity, or they have no natural \*\*\*ketolase\*\*\* activity and this is supplied by (4), particularly expressed from a transgene. They may also have some beta-cyclase activity, which is increased by expressing (2), or no such activity, which is then imparted by (2). (A) may also have increased (or

imparted) activity of (a) hydroxylase (particularly as a 314 aa sequence (6)) and/or (b) a wide range of other enzymes involved in biosynthesis of carotenoids, e.g. 1-deoxy-D-xylose-5-phosphate synthase; isopentyl diphosphate-DELTA-isomerase; (geranyl-)geranyl-diphosphate synthase; phytoene synthase or desaturase, or beta-carotene desaturase. Suitable organisms are those that produce carotenoids (either naturally or as a result of metabolic complementation or regulation), e.g. bacteria, yeast, algae, fungi or most preferably plants. Many suitable plants are listed, e.g. *Tagetes erecta*; *T. patula*; *Adonis*; *Crocus*; *Genista*; *Medicago*; *Rosa*; and *Zinnia*. Preferred Materials: (2) and (6) are from tomato and (4) is from *Haematococcus pluvialis*. The specification includes nucleic acid sequences that encode these enzymes. Specified (I) are astaxanthin (most preferred); \*\*\*canthaxanthin\*\*\*; echinenone; 3- or 3'-hydroxyechinenone; adonirubin and adonixanthin.

USE - (A) are useful as foods or animal feeds, or for preparation of (I)-containing extracts for use as food or feed supplements (claimed). (I) are natural antioxidants and pigments (e.g. for fish).

ADVANTAGE - Increasing the activities of \*\*\*ketolase\*\*\* and beta-cyclase provides high yields of (I), specifically astaxanthin, without extensive formation of hydroxylated by-products.

EXAMPLE - The vector pMKP1 provides flower-specific expression of (i) the chromoplast-specific lycopene beta-cyclase of tomato, under control of promoter P76 and (ii) \*\*\*ketolase\*\*\* NP196 from *Nostoc punctiforme* ATCC 29133, under control of the EPSPS promoter. P76 was amplified from genomic DNA of *Arabidopsis thaliana*, the product cloned as an EcoRV fragment into pSUN5 to form p76 into which (a) the 35S terminator and (b) the B-gene, amplified from tomato, were inserted to form pB. This was cut with PmeI and SspI and the fragment (3906 bp) cloned into vector MSP108 (containing a cassette which included the EPSPS promoter, sequence for the pea *rbcS* transit peptide, the sequence for \*\*\*ketolase\*\*\* NP196, and the ocs terminator and polyadenylation sequence), to form pMKP1. This vector was used to generate transgenic *Tagetes* by *Agrobacterium*-mediated transformation, but no figures for production of carotenoids in the resulting plants are given. (357 pages)

L5 ANSWER 12 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2005-10840 BIOTECHDS <<LOGINID::20060806>>

TITLE: Preparation of ketocarotenoids, useful in foods and animal feeds, by growing genetically modified organism, particularly plant, having altered ketolase activity; involving vector-mediated ketolase gene transfer expression in host cell

AUTHOR: SAUER M; SCHOPFER C R; FLACHMANN R; HERBERS K; KUNZE I; KLEBSATTEL M; LUCK T; VOESTE D; PFEIFFER A; TSCHOEP H

PATENT ASSIGNEE: SUNGENE GMBH and CO KGAA

PATENT INFO: WO 2005019461 3 Mar 2005

APPLICATION INFO: WO 2004-EP8625 31 Jul 2004

PRIORITY INFO: DE 2004-102004007624 17 Feb 2004; WO 2003-9101 18 Aug 2003

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 2005-202658 [21]

AN 2005-10840 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - Method for preparing ketocarotenoids (I) by growing a genetically modified \*\*\*organism\*\*\* (A) which, compared with the wild type, has altered activity of a \*\*\*ketolase\*\*\* (II), where this alteration is produced by any of the amino acid (aa) sequences (2; 260 aa); (10; 253 aa); (12; 253 aa) or (14; 256 aa), all reproduced, or their derivatives with at least 80%, 90%, 90% and 50% identity, respectively, formed by substitution, deletion or insertion.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) (A) in which \*\*\*ketolase\*\*\* activity is increased (or provided, if not already present) by expressing one or more of the amino acid sequences specified above; (2) ketolases of sequences (2), (10), (12) and their derivatives as specified; and (3) nucleic acid that encodes the ketolases of item (2).

BIOTECHNOLOGY - Preferred Organisms: These already have some \*\*\*ketolase\*\*\* activity, and this is increased by expression of the specified sequences, or they have no natural \*\*\*ketolase\*\*\* activity and this is imparted by the specified sequences, particularly as a

transgene. They may also show increased (or imparted) activity of (a) hydroxylase and/or beta-cyclase or (b) a wide variety of other enzymes involved in biosynthesis of carotenoids, e.g. isopentyl-diphosphate-DELTA-isomerase; (geranyl-)geranyl-diphosphate synthase; phytoene synthase or desaturase, or approximately carotene desaturase. Suitable organisms are those that produce carotenoids (either naturally or after metabolic complementation or regulation), e.g. bacteria, yeast, algae, fungi or most preferably plants, particularly where the highest level of

\*\*\*ketolase\*\*\* expression is in the flowers (particularly by using a flower-specific promoter to control the gene for \*\*\*ketolase\*\*\*). The plants preferably also have reduced activity of epsilon-cyclase (e.g. as a result of introducing antisense RNA or a ribozyme), and include chromoplasts in the petals. Many suitable plants are listed, e.g. *Tagetes erecta*; *T. patula*; *Adonis*; *Crocus*; *Genista*; *Medicago*; *Rosa*; and *Zinnia*. Preferred Materials: \*\*\*Ketolase\*\*\* (2) is from *Nodularia spumigena*; (10) and (12) from *Nostoc punctiforme* and (14) from *Gloeobacter violaceus*, and the specification includes the nucleic acid sequences that encode these enzymes. Specified (I) are astaxanthin (most preferred); \*\*\*canthaxanthin\*\*\*; echinenone; 3- or 3'-hydroxyechinenone; adonirubin and adonixanthin.

USE - (A) are useful as foods or animal feeds, or for preparation of (I)-containing extracts for use as food or feed supplements (claimed). (I) are natural antioxidants and pigments (e.g. for fish).

ADVANTAGE - The specified ketolases provide high yields of (I), specifically astaxanthin, without extensive formation of hydroxylated by-products.

EXAMPLE - The vector pNSO37:BKT was prepared from pCR2.1-TOPO by inserting an 807 bp sequence that encodes the \*\*\*ketolase\*\*\* from *Nodularia spumigena* CCAUV 01-037. It was used, together with plasmid pMCL-CrYIBZ/idi/gps (containing several other genes involved in biosynthesis of carotenoids) to transform *Escherichia coli* TOP10. The transformants were cultured overnight then carotenoid contents (in ng/ml) of the culture measured: 2190 total carotenoids; 1230 total ketocarotenoids; 1038 astaxanthin; 457 zeaxanthin; 285 beta-carotene; 218 beta-cryptoxanthin; 148 adonixanthin and 45 \*\*\*canthaxanthin\*\*\*. (317 pages)

L5 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2004:292102 CAPLUS <<LOGINID::20060806>>

DOCUMENT NUMBER: 140:302431

TITLE: Production of canthaxanthin by recombinant *Phaffia rhodozyma*

INVENTOR(S): Hoshino, Tatsuo; Ojima, Kazuyuki; Setoguchi, Yutaka

PATENT ASSIGNEE(S): DSM Ip Assets B.V., Neth.

SOURCE: PCT Int. Appl., 13 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004029261	A2	20040408	WO 2003-EP10294	20030916
WO 2004029261	A3	20040527		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003267371	A1	20040419	AU 2003-267371	20030916
EP 1543132	A2	20050622	EP 2003-748041	20030916
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2006500046	T2	20060105	JP 2004-538914	20030916



CN 1788091 A 20060614 CN 2003-825474 20030916  
 US 2006141557 A1 20060629 US 2006-528846 20060209  
 PRIORITY APPLN. INFO.: EP 2002-21600 A 20020927  
 WO 2003-EP10294 W 20030916

AB Disclosed is a process for producing \*\*\*canthaxanthin\*\*\* and echinenone which comprises cultivating a recombinant \*\*\*microorganism\*\*\* which is expressing a .beta.-carotene \*\*\*ketolase\*\*\* gene and belonging to the genus Xanthophyllomyces ( \*\*\*Phaffia\*\*\* ) in an aq. nutrient medium under aerobic conditions, and isolating the resulted carotenoids from the cells of said recombinant \*\*\*microorganism\*\*\* or from the cultured broth. Thus, the crtW gene from Alcaligenes strain PC-1 which encodes .beta.-carotene ketolase was cloned into Phaffia rhodozyma ATCC 96815.

L5 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 2004:587901 CAPLUS <<LOGINID::20060806>>

DOCUMENT NUMBER: 141:135219

TITLE: Method for producing ketocarotenoids using organisms with altered .beta.-carotene ketolase activity

PATENT ASSIGNEE(S): BASF AG, Germany

SOURCE: Ger. Offen., 43 pp.

CODEN: GWXXBX

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 10300649	A1	20040722	DE 2003-10300649	20030109
CA 2512151	AA	20040729	CA 2003-2512151	20031224
WO 2004063366	A1	20040729	WO 2003-EP14876	20031224
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003294001	A1	20040810	AU 2003-294001	20031224
EP 1585813	A1	20051019	EP 2003-789415	20031224
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN 1735686	A	20060215	CN 2003-80108484	20031224
JP 2006512914	T2	20060420	JP 2004-566030	20031224
WO 2004063359	A2	20040729	WO 2004-EP99	20040109
WO 2004063359	A3	20050127		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ				
WO 2004063358	A1	20040729	WO 2004-EP100	20040109
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ				
EP 1592783	A2	20051109	EP 2004-700978	20040109
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
EP 1592784	A1	20051109	EP 2004-700993	20040109
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN 1759173	A	20060412	CN 2004-80006379	20040109
CN 1759174	A	20060412	CN 2004-80006380	20040109
JP 2006513729	T2	20060427	JP 2005-518517	20040109
JP 2006515516	T2	20060601	JP 2005-518516	20040109
NO 2005003206	A	20050830	NO 2005-3206	20050630

US 2006053513 A1 20060309 US 2005-541513 20050708  
 US 2006099670 A1 20060511 US 2005-541993 20050708  
 PRIORITY APPLN. INFO.: DE 2003-10300649 A 20030109  
 DE 2003-10341271 A 20030908  
 DE 2003-10341272 A 20030908  
 WO 2003-EP14876 W 20031224  
 WO 2004-EP100 W 20040109  
 WO 2004-EP99 W 20040109

AB The present invention concerns a procedure for the prodn. of ketocarotinoids through cultivation of genetically altered organisms which contain, in comparison to the wild type, an altered .beta.-carotene ketolase activity, the genetically altered organisms as well as their use as food and animal feed and for the prodn. of ketocarotinoid exts. Thus, the .beta.-carotene ketolase genes of Nostoc punctiforme strain PCC73102 were cloned and sequenced. These genes were expressed in .beta.-carotene-producing E. coli. These transgenic bacteria produced echinenon and canthaxanthin. Expression of these genes in zeaxanthin-producing E. coli resulted in prodn. of astaxanthin.

L5 ANSWER 15 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
 ACCESSION NUMBER: 2004-18430 BIOTECHDS <<LOGINID::20060806>>

TITLE: Preparing genetically modified Blakeslea, useful for preparation of carotenoids, useful as food additives, cosmetics or pharmaceuticals, comprises transformation, optional homokaryotizing, and selection; recombinant fungus useful for carotenoid production for use in the pharmaceutical industry

AUTHOR: MATUSCHEK M; HEINEKAMP T; SCHMIDT A; BRAKHAGE A

PATENT ASSIGNEE: BASF AG

PATENT INFO: WO 2004063358 29 Jul 2004

APPLICATION INFO: WO 2004-EP100 9 Jan 2004

PRIORITY INFO: DE 2003-1041272 8 Sep 2003; DE 2003-1000649 9 Jan 2003

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 2004-544087 [52]

AN 2004-18430 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - Preparing a genetically modified \*\*\*organism\*\*\* (A) of the genus Blakeslea comprises first transforming at least one cell; then optionally homokaryotizing the cells so that cells are produced in which the nuclei are all simultaneously altered in one or more genetic characteristics and these alterations are expressed; and finally selection and culture of the modified cell(s).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) genetically altered, multinuclear cells of Blakeslea, especially B. trispora, produced by the new method; (2) two new promoters, of (2160 bp and 1520 bp, sequences fully defined in the specification) for use in the new method; (3) a terminator (774 bp, sequence fully defined in the specification), for use in the new method; and (4) a vector (15739 bp, sequence fully defined in the specification) for use in the new method.

BIOTECHNOLOGY - Preferred Fungus: The fungus is preferably B. trispora. Preferred Process: The initial transformation is with a vector or free nucleic acid, particularly where the vector can integrate into the genome and includes a promoter and/or terminator; also a resistance gene. Transformation is by using Agrobacterium; conjugation; electroporation; DNA particle bombardment or other standard methods. Homokaryotization is done with a mutagenic agent (II) and selection is by labeling and/or by choice of mononuclear cells. Preferably selection is done with 5-carba-5-deaza-riboflavin and hygromycin (hyg) or with a mixture of 5-fluoro-orotate, uracil and hyg. Preferred Materials: The vector for transformation contains a gpd (specifically sequence (1)), carB, carA and/or tefl (specifically sequence (35)) promoter and/or a trpC terminator (specifically sequence (2)). Preferably both gpd and trpC are from Aspergillus nidulans, and the most preferred vector is (3). The resistance gene is particularly the Escherichia coli hph (hygromycin resistance) gene and (II) is particularly N-methyl-N'-nitro-nitrosoguanidine, UV light or X-rays. The vector also includes the information needed for preparation of carotenoids or their precursors, especially carotenes and xanthophylls; optionally also the information required for expression of \*\*\*ketolase\*\*\* and/or hydroxylase

activity. The vector may also be designed to switch off genetic information in the cell, especially the phytoene desaturase gene or the lycopene cyclase gene. The specification lists several sequences for hydroxylase genes (most preferably from *Haematococcus pluvialis*, *Erwinia uredo* or *Thermus thermophilus*, also a sequence encoding a

\*\*\*ketolase\*\*\* (from *Nostoc* sp. PCC73102). Preferred Carotenoids: 16 compounds are specified, e.g. astaxanthin; zeaxanthin; (3-hydroxy)echinenone; \*\*\*canthaxanthin\*\*\*; lycopene; alpha- or beta-carotene or lycopene.

USE - (A) are used for production of carotenoids, particularly carotenes and xanthophylls, useful as animal and human nutrients, or supplements, cosmetics and pharmaceuticals, particularly for pigmentation or coloring beverages, but also as antioxidants.

ADVANTAGE - Genetic modification makes possible production, in *Blakeslea* of xanthophylls, which are not normally produced. The new method provides multinucleated cells with stable genetic modifications.

EXAMPLE - Vector pBinAhygBTpTEF1-HPcrtZ (17877 bp sequence reproduced) includes, between T-DNA border sequences, the hydroxylase (HPcrtZ) gene from *Haematococcus pluvialis* under control of the tef1 promoter of *Blakeslea trispora*; the hph (hygromycin resistance) gene and the gpdA promoter. It was used to transform *B. trispora* by *Agrobacterium*-mediated transfer, then hygromycin-resistant clones selected on potato-glucose agar. Selected cells were grown, harvested, lysed, extracted with tetrahydrofuran, and the extract residue was analyzed by HPLC. The chromatogram indicated presence of zeaxanthin, as well as beta-carotene and lycopene. (459 pages)

L5 ANSWER 16 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2004-18987 BIOTECHDS <<LOGINID::20060806>>

TITLE: Production of ketocarotenoids with low hydroxylated  
by-product content, for use e.g. in pigmenting feedstuffs, by  
culturing genetically modified organisms having modified  
ketolase activity;  
ketocarotenoid production via plasmid expression in host  
cell for use in food

AUTHOR: SAUER M; FLACHMANN R; KLEBSATTEL M; SCHOPFER C R

PATENT ASSIGNEE: SUNGENE GMBH and CO KGAA

PATENT INFO: DE 10253112 3 Jun 2004

APPLICATION INFO: DE 2002-1053112 13 Nov 2002

PRIORITY INFO: DE 2002-1053112 13 Nov 2002; DE 2002-1053112 13 Nov 2002

DOCUMENT TYPE: Patent

LANGUAGE: German

OTHER SOURCE: WPI: 2004-489014 [47]

AN 2004-18987 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - Production of ketocarotenoids (I) involves culturing genetically modified organisms having modified \*\*\*ketolase\*\*\* (KLA) activity (compared with wild strains) due to a \*\*\*ketolase\*\*\* (II) containing a specific sequence (A) of 258 aminoacids (given in the specification as SEQ. ID. NO. 2) or a mutant sequence of (A), provided that (A') has at least 42% homology with (A).

DETAILED DESCRIPTION - Production of ketocarotenoids (I) involves culturing genetically modified organisms having modified \*\*\*ketolase\*\*\* (KLA) activity (compared with wild strains) due to a \*\*\*ketolase\*\*\* (II) containing a specific sequence (A) of 258 aminoacids (given in the specification as SEQ. ID. NO. 2) or a sequence (A) derived from (A) by substitution, insertion or deletion of amino acids, provided that (A') has at least 42% homology with (A). INDEPENDENT CLAIMS are included for: (1) genetically modified organisms which: (a) show increased KLA activity compared with wild strains (or into which KLA activity is introduced if the wild strain has no KLA activity), having KLA activity due to (II); and/or (b) contain at least one transgenic nucleic acid encoding (A) or (A') or at least two endogenous nucleic acid sequences encoding (II); (2) new ketolases (II'), which contain: (a) a specific sequence (Ai) of 262 aminoacids (SEQ. ID. NO. 8) or a sequence (Ai') derived from (Ai) by substitution, insertion or deletion, provided that (Ai') has at least 70% homology with (Ai) and that a specific sequence of 262 aminoacids (SEQ. ID. NO. 4) is excluded; (b) a specific sequence (Aii) of 253 aminoacids (SEQ. ID. NO. 6) or a sequence (Aii') derived from (Aii) by substitution, insertion or deletion, provided that (Aii') has at least 70% homology

with (Aii); (c) a specific sequence (Aiii) of 253 aminoacids (SEQ. ID. NO. 12) or a sequence (Aiii') derived from (Aiii) by substitution, insertion or deletion, provided that (Aiii') has at least 70% homology with (Aiii) and that SEQ. ID. NO. 4 is excluded; or (d) a specific sequence (Aiv) of 267 aminoacids (SEQ. ID. NO. 49) or a sequence (Aiv') derived from (Aiv) by substitution, insertion or deletion, provided that (Aiv') has at least 50% homology with (Aiv) and that a specific sequence of 267 aminoacids (SEQ. ID. NO. 47) is excluded, where all the sequences are defined in the specification; (3) nucleic acids encoding (II'), provided that a specific sequence of 762 bases (SEQ. ID. NO. 5; sequence defined in the specification) is excluded; and (4) the use as

\*\*\*ketolase\*\*\* of proteins which contain SEQ. ID. NO. 4 (or a derived sequence having at least 70% homology with SEQ. ID. No. 4), SEQ. ID. NO. 6 (or a derived sequence having at least 65% homology with SEQ. ID. No. 6) or SEQ. ID. NO. 47 (or a derived sequence having at least 50% homology with SEQ. ID. No. 47) and show KLA activity, where all the sequences are defined in the specification.

**BIOTECHNOLOGY - Preferred Organisms:** The starting microorganisms produce carotenoids (naturally or by genetic supplementation), and are specifically microorganisms (especially bacteria, yeasts, algae or fungi) or plants. Specified in the claims are 23 preferred types of microorganisms (e.g. *Escherichia*, *Flavobacterium*, *Nostoc*, *Synechocystis*, *Hansenula*, *Fusarium* and *Dunaliella*); 28 preferred families of plants (e.g. *Ranunculaceae*, *Cannabaceae*, *Brassicaceae*, *Amaranthaceae*, *Solanaceae* and *Lamiaceae*); and about 100 preferred genera of plants (e.g. *Acacia*, *Calendula*, *Gentiana*, *Helianthus*, *Linum*, *Rhododendron*, *Spartium* and *Zinnia*). **Preferred Process:** Nucleic acids encoding (II) are introduced into the host organisms, preferably nucleic acids containing a specific sequence of 777 bases (SEQ. ID. NO. 1) from *Nostoc* sp. strain PCC7120. The modified microorganisms additionally show elevated hydroxylase and/or beta-cyclase activity, preferably due to expression of at least one nucleic acid encoding hydroxylase and/or beta-cyclase, especially using: (a) a nucleic acid encoding a hydroxylase having specific sequence of 322 amino acids (SEQ. ID. NO. 16) (or a derived sequence having at least 20% homology), the nucleic acid preferably having a specific sequence of 1608 bases (SEQ. ID. NO. 15) from *Haematococcus pluvialis*; and/or (b) a nucleic acid encoding a beta-cyclase having specific sequence of 500 aminoacids (SEQ. ID. NO. 18) (or a derived sequence having at least 20% homology), the nucleic acid preferably having a specific sequence of 1650 bases (SEQ. ID. NO. 17) from *Lycopersicon esculentum*. All sequences are defined in the specification. The genetically modified \*\*\*organism\*\*\* is cultured, the \*\*\*organism\*\*\* is harvested and (I) is recovered from the product.

**USE - (I)** are natural antioxidants and pigments, especially useful (particularly in the case of (Ia)) as pigmenting additives in animal feed, specifically feed for trout, salmon or shrimps. The use of the (I)-producing genetically modified organisms (specifically microorganisms or plants) is claimed as feedstuffs or foodstuffs, in the production of (I)-containing extracts or for producing feed or food supplements.

**ADVANTAGE -** The process provides large amounts of (I) having a low content of hydroxylated by-products, especially in the case of (Ia).

**EXAMPLE -** DNA encoding the whole primary \*\*\*ketolase\*\*\* sequence from *Nostoc* sp. strain PCC7120 was isolated, amplified by PCR and used to produce a plasmid pNOSTF-G. A plasmid pMCL-Crt-YIBZ/idi/gps, for the synthesis of zeaxanthin in *Escherichia coli*, was constructed in 3 stages via the intermediate stages pMCL-CrtYIBZ and pMCL-CrtYIBX/idi, using the high copy number plasmid vector pMCL200. *Escherichia coli* strain TOP10 was transformed with the plasmids pNOSTF-G and pMCL-Crt-YIBZ/idi/gps to give carotenoid producing strain, which provided a culture supernatant containing 491 ng/ml astaxanthin (Ia), 186 ng/ml adonirubin and 120 ng \*\*\*canthaxanthin\*\*\* (i.e. the required ketocarotenoid (Ia) as main product). For comparison, a supernatant obtained using a strain producing a \*\*\*ketolase\*\*\* from *Haematococcus pluvialis* gave a culture supernatant containing 13 ng/ml (Ia), 102 ng/ml adoxanthin and 120 ng zeaxanthin (i.e. the hydroxylated by-product zeaxanthin as main product).(101 pages)

TITLE: New isolated nucleic acid encoding carotenoid ketolase enzyme, useful for producing cyclic ketocarotenoid compounds such as adonirubin, echinenone, and as probes or primers to identify nucleic acids encoding the enzyme; vector-mediated carotenoid-ketolase gene transfer and expression in host cell for recombinant protein production

AUTHOR: CHENG Q; TAO L

PATENT ASSIGNEE: DU PONT DE NEMOURS and CO E I

PATENT INFO: WO 2003012056 13 Feb 2003

APPLICATION INFO: WO 2002-US24317 1 Aug 2002

PRIORITY INFO: US 2001-309653 2 Aug 2001; US 2001-309653 2 Aug 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-300493 [29]

AN 2003-13363 BIOTECHDS <<LOGINID::20060806>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid encoding a carotenoid \*\*\*ketolase\*\*\* enzyme, which: (i) encodes an amino acid sequence containing all six conserved motifs of CrtO enzymes of *Rhodococcus erythropolis* AN12 strain, *Deinococcus radiodurans* R1 strain, and *Synechocystis* sp. PCC6803 strain; (ii) encodes a sequence of 532 amino acids, given in specification; or (iii) hybridizes to (i) or (ii), is new.

DETAILED DESCRIPTION - A new isolated nucleic acid is an: (i) isolated nucleic acid molecule encoding an amino acid sequence containing all 6 conserved motif sequences of CrtO enzymes of *Rhodococcus erythropolis* AN12 strain, *Deinococcus radiodurans* R1 strain, and *Synechocystis* sp. PCC6803 strain such as S1 - S6; (ii) an isolated nucleic acid encoding a sequence of 532 amino acids (S1), given in the specification; or (iii) an isolated nucleic acid that hybridizes with (i) or (ii), or an isolated nucleic acid complementary to (i), where the nucleic acid is not the sequence of a crtO gene from *Synechocystis* sp. PCC6803 strain having a sequence of 1629 nucleotides (S3), given in the specification, or is not the sequence of a crtO gene from *Deinococcus radiodurans* R1 strain having a sequence of 1536 nucleotides (S4), given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) a polypeptide (II) encoded by (I); (2) an isolated nucleic acid (III) comprising a sequence encoding a carotenoid \*\*\*ketolase\*\*\* enzyme of 532 amino acids that has 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having (S1), or a second nucleotide sequence comprising the complement of the first nucleotide sequence; (3) an isolated nucleic acid (IV) encoding a carotenoid \*\*\*ketolase\*\*\* enzyme having 70 % identity based on the Smith-Waterman method of alignment to all of the amino acid sequences defining the CrtO diagnostic motifs, provided the isolated nucleic acid is not (S3) or (S4); (4) a polypeptide (V) encoded by (IV) provided that the polypeptide is not a sequence of 542 (S5) or 511 (S6) amino acids, given in the specification; (5) a chimeric gene (VI) comprising (I), (III) or (IV) operably linked to suitable regulatory sequences; (6) a transformed host cell (VII) comprising (VI); (7) obtaining a nucleic acid encoding a carotenoid \*\*\*ketolase\*\*\* enzyme comprising: (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence chosen from (S1) and (S4); and (b) amplifying an insert present in a cloning vector using the oligonucleotide primer, where the amplified insert encodes a carotenoid \*\*\*ketolase\*\*\* enzyme; (8) a product of (7); (9) a mutated gene encoding a carotenoid \*\*\*ketolase\*\*\* enzyme having an altered biological activity produced by: (a) digesting a mixture of nucleotide sequences with restriction endonucleases where the mixture comprises a native carotenoid \*\*\*ketolase\*\*\* gene, a first population of nucleotide fragments which will hybridize to the native carotenoid \*\*\*ketolase\*\*\* gene, and a second population of nucleotide fragments which will not hybridize to the native carotenoid \*\*\*ketolase\*\*\* gene, where a mixture of restriction fragments are produced; (b) denaturing the mixture of restriction fragments; (c) incubating the denatured mixture of restriction fragments of (b) with a polymerase; and (d) repeating (b) and (c) where a mutated carotenoid \*\*\*ketolase\*\*\* gene is produced encoding a protein having an altered biological activity. Xaa1-Met-Ser-Xaa2-Asp-Gln-Met-Xaa3 (S1) Xaa1 = Asp or Glu; Xaa2 = Phe or Leu; and Xaa3 = Met or Phe. Tyr-Leu-Thr-Gly-Ala-Xaa-Thr-His-Pro (S2) Xaa = Ser or Gly. Xaa1-Gly-Leu-Xaa2-Tyr-Xaa3-Xaa4-Xaa5-Asp-Pro (S3) Xaa1 = His or Tyr; Xaa2 = Arg, His or Glu; Xaa3 = Ile or

Leu; Xaa4 = Asp, Glu or Phe; and Xaa5 = Cys or Val. His-Asn-Xaa1-Leu-Val-Xaa2-Ala-Ala-Tyr (S4) Xaa1 = Ala or Gly; and Xaa2 = Ser, Thr or Cys. Glu-Xaa1-Phe-Xaa2-Xaa3-Glu-Xaa4-Xaa5-Xaa6-Ala (S5) Xaa1 = Tyr or Trp; Xaa2 = Asp or Ser; Xaa3 = Ser or Glu; Xaa4 = Arg or Ala; Xaa5 = Val or Leu; and Xaa6 = Lys or Arg. Tyr-Xaa1-Xaa2-Phe-Xaa3-Xaa4-Xaa5-Trp (S6) Xaa1 = Arg or Gly; Xaa2 = Arg or Gln; Xaa3 = Val or Leu; Xaa4 = Ala, Asp or Asn; and Xaa5 = Asp, Val or Tyr.

BIOTECHNOLOGY - Preferred Polypeptide: (II) comprises a sequence of (S1). Preferred Host Cell: (VII) is a bacteria, yeast, filamentous fungi, algae or green plant cell. Preferably, the host cell is chosen from *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Erythrobacter*, *Chlorobium*, *Chromatium*, *Flavobacterium*, *Cytophaga*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacterium*, *Mycobacterium*, *Deinococcus*, *Escherichia*, *Erwinia*, *Pantoea*, *Pseudomonas*, *Sphingomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylobacterium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, *Myxococcus*, *Spirulina*, *Haemotacoccus*, or *Dunaliella*. The host cell is optionally chosen from soybean, rapeseed, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops, sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees and forage grasses.

USE - (I), Or another nucleic acid (III and IV) encoding the enzyme, is useful for obtaining a nucleic acid encoding a carotenoid

\*\*\*ketolase\*\*\* enzyme by: (a) probing a genomic library with (I), (III) or (IV); (b) identifying a DNA clone that hybridizes with the nucleic acid molecule; and (c) sequencing the genomic fragment that comprises the clone identified in (b), where the sequenced genomic fragment encodes a carotenoid \*\*\*ketolase\*\*\* enzyme. (I), (III) Or (IV) is also useful for producing cyclic ketocarotenoid compounds, by: (a) providing a host cell which produces monocyclic or bicyclic carotenoids such as beta-carotene, gamma-carotene, zeaxanthin, rubixanthin, echinenone or torulene; (b) transforming the host cell with (I), (III) or (IV) encoding a carotenoid \*\*\*ketolase\*\*\* enzyme; and (c) growing the transformed host cell under conditions whereby a cyclic ketocarotenoid such as \*\*\*canthaxanthin\*\*\*, astaxanthin, adonixanthin, adonirubin, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, 4-keto-gamma-carotene, 4-keto-rubixanthin, 4-keto-torulene, 3-hydroxy-4-keto-torulene, deoxyflexixanthin, myxobactone, is produced. The transformed host cell is chosen from *Aspergillus*, *Trichoderma*, *Haemotacoccus*, *Dunaliella*, etc. The host cell is optionally chosen from soybean, rapeseed, sunflower, cotton, etc. (I) Encoding conserved motifs is useful for obtaining a nucleic acid encoding a carotenoid \*\*\*ketolase\*\*\* enzyme by: (a) providing nucleic acid probes encoding CrtO diagnostic motif sequences; (b) identifying a DNA clone that hybridizes with all of the probes; and (c) sequencing the genomic fragment that comprises the clone identified in (b), where the sequenced genomic fragment encodes a carotenoid \*\*\*ketolase\*\*\* enzyme. A chimeric gene (VI) is useful for regulating cyclic ketocarotenoid biosynthesis in an \*\*\*organism\*\*\* by introducing (VI) into a host cell and growing the host cell under conditions whereby the carotenoid \*\*\*ketolase\*\*\* gene is expressed and the cyclic ketocarotenoid biosynthesis is regulated. The regulation may be upregulation of cyclic ketocarotenoid biosynthesis, where the carotenoid \*\*\*ketolase\*\*\* gene is overexpressed on a multicopy plasmid or is operably linked to a inducible or regulated promoter. Optionally the cyclic ketocarotenoid biosynthesis may be down regulated, where the carotenoid \*\*\*ketolase\*\*\* gene is expressed in antisense orientation or is disrupted by insertion of foreign DNA into the coding region (all claimed).

EXAMPLE - An environmental sample containing *Rhodococcus erythropolis* AN12 strain was obtained from a waste water treatment facility. One ml of activated sludge was inoculated directly into 10 ml of S12 medium. Aniline was used as the sole source of carbon and energy. The culture was maintained by addition of 100 parts per million (ppm) aniline every 2 - 3 days. The culture was diluted (1:100 dilution) every 14 days. Bacteria that utilize aniline as a sole source of carbon and energy were further isolated and purified on S12 agar. When 16s rRNA gene

of AN12 was sequenced and compared to other 16s rRNA sequence in the GenBank (RTM) sequence database, the 16s rRNA gene of AN12 strain had 98 % similarity to the 16s rRNA gene sequences of high G+C gram positive Rhodococcus genus. Genomic nucleotide sequences were isolated from Rhodococcus genus AN12 strain and compared to genes from existing database. There were two open reading frames (ORFs) that shared homology with two different putative phytoene dehydrogenases. The gene in ORF 1 was designated as crtO and the other was designated as crtI. The two genes shared very little homology with each other (24 % identity). Sequence in ORF 1 (1599 nucleotides) had 35 % identity with a gene suspected to be a phytoene dehydrogenase from Deinococcus radiodurans. CrtI, but not CrtO, was determined to be dehydrogenase since the crtI mutant with intact crtO exhibited the phytoene dehydrogenase knockout phenotype. crtO (ORF1) encoded a \*\*\*ketolase\*\*\* that adds ketone groups to the beta-ionone rings of the cyclic carotenoids to produce ketocarotenoids. Previously two types of carotenoid ketolases (the CrtW type and the CrtO type) were reported Kajiwara, et al., 1995, Plant Mol. Biol. 29:343-352; Fernandez-Gonzalez, et al., J.Biol.Chem.,1997, 272:9728-9733. All CrtW enzymes were symmetric 2-ring ketolases from AN12 and Deinococcus were symmetric 2-ring ketolases, similar to CrtW. The CrtW type \*\*\*ketolase\*\*\* symmetrically adds a ketone group to both beta-ionone rings of beta-carotene to generate \*\*\*canthaxanthin\*\*\*. CrtO isolated from Synechocystis sp. PCC6803 was shown to be a new type of asymmetrically acting beta-carotene \*\*\*ketolase\*\*\* that introduces a keto group to only one of the beta-ionone rings of beta-carotene to generate echinenone. The Synechocystis CrtO (slr0088) had significant homology to the bacterial phytoene dehydrogenases but showed no such activity biochemically. The CrtO gene was isolated from Rhodococcus erythropolis AN 12 and was 532 amino acids in length. The most similar sequence to the Rhodococcus crtO as determined by the Basic Local Alignment Search Tool (BLAST) program was the 511 amino acid protein isolated from Deinococcus with the putative function of phytoene dehydrogenase DR0093. The second closest alignment generated from the BLAST search to the Rhodococcus CrtO was to a Synechocystis hypothetical protein (slr0088) having 542 amino acids, that was later confirmed to be a CrtO \*\*\*ketolase\*\*\*. The CrtO from Rhodococcus had 35 % amino acid identity and 64 % similarity with the CrtO from Synechocystis. It shared very little sequence homology with the CrtW type of enzymes. Phylogenetic analysis grouped with the Rhodococcus CrtO, the Deinococcus CrtO and the Synechocystis CrtO together in a separate branch, separate from all the CrtW enzymes. The CrtO designation of the Rhodococcus ORF was based on the shared sequence homology with the Synechocystis CrtO. Motif analysis was performed using the Multiple Expectation maximization for Motif Elicitation (MEME) program. Six conserved motifs were identified in each of the three CrtO enzymes. The location of the motifs was also conserved in the CrtO enzymes compared. The consensus sequence of the motifs was used to search the European Molecular Biology Laboratory (EMBL) and SwissProt (RTM) databases using the MAST program. No other proteins in the public databases were found to have all six motifs. (90 pages)

=> d his

L1 QUE ((BETA-CAROTENE ADJ KETOLASE) OR (CAROTENE ADJ KETOLASE) OR  
L2 604 S L1  
L3 77 S (MICROORGANISM OR ORGANISM OR PHAFFIA) (S) L2  
L4 23 S CANTHAXANTHIN(S)L3  
L5 17 DUP REM L4 (6 DUPLICATES REMOVED)

=> log y